THE USE OF MICROFOCUS COMPUTERIZED TOMOGRAPHY AS A NEW TECHNIQUE FOR CHARACTERIZING BONE TISSUE AROUND ORAL IMPLANTS

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Qualitative and quantitative analysis of peri-implant tissues around retrieved oral implants is typically done by means of light microscopy on thin histological sections containing the metal surface and the undecalcified bone. It remains, however, a labor-intensive and thus time-consuming job. Moreover, it is a destructive technique that allows tissue quantification in only a limited number of two-dimensional sections. As an alternative, we evaluated the bone structure around screw-shaped titanium implants by means of microfocus computerized tomography (micro-CT) because it presents a number of advantages compared to conventional sectioning techniques: micro-CT is nondestructive, fast, and allows a fully three-dimensional characterization of the bone structure around the implant. Images can be reconstructed in an arbitrary plane, and three-dimensional reconstructions are also possible. Because of its high resolution, individual trabeculae can be visualized. The accuracy of micro-CT was qualitatively evaluated by comparing histological sections with the corresponding CT slices for the same specimen. The overall trabecular structure is very similar according to both techniques. Even very close to the interface, the titanium implant does not seem to produce significant artifacts. Furthermore, because the complete digital data on the trabecular bone structure around the implant is available, it is possible to create finite-element models of the bone-implant system that model the trabeculae in detail so that mechanical stress transfer at the interface can be studied at the level of individual trabeculae. Therefore, micro-CT seems to be very promising for the *in vitro* assessment of the three-dimensional bone structure around oral implants. Further research will be needed to evaluate its accuracy in a more quantitative way.

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INTRODUCTION

Qualitative and quantitative measurements of the peri-implant tissues around retrieved implants are typically done by means of light microscopy on thin histological sections containing both the undecalcified bone tissue and the metal implant. It is very important to preserve the original bone–implant interface so that no relevant information will be lost. Different techniques exist to prepare thin sections from plastic-embedded implant-containing specimens: a ''sawing and grinding'' technique, as developed by Donath and Breuner,[1] and a ''modified sawing mi-

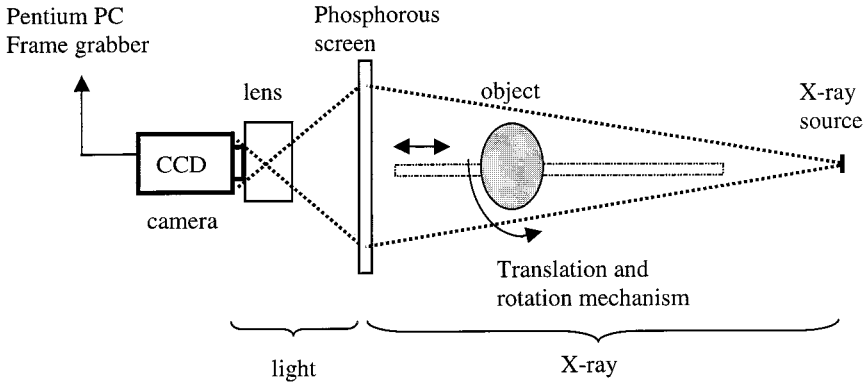


FIGURE 1. Schematic overview of the micro-CT system.

crotome'' technique, as developed by van der Lubbe and Klein.[2,3] In the first technique, a section between 50–200 μm thick is cut by means of a precision-guided, diamond-coated band saw. This section is then thinned down to a thickness of 5 to 10 μm by means of an automatic grinding machine. In the second technique, 5- to 10-μm-thick sections are directly sawed by means of a horizontal-rotation–sawing microtome with a diamond-edge blade. The sections can be stained prior to light microscopy.

With these techniques, it is not possible to perform a fully three-dimensional histological analysis around the implant because of the limited two-dimensional sections that can be prepared from one specimen. Even when a maximum number of sections are taken from one specimen, a considerable amount of material will be lost, for example because of the thickness of the sawing blade. Moreover, the techniques are labor-intensive and thus time-consuming. Besides, the technique is destructive, so that other research with respect to the intact bone–implant interface (*eg*, mechanical testing of the interface strength) is impossible. Therefore, we have applied microfocus computerized tomography (micro-CT)[4] as a new technique to analyze bone–implant specimens. It is a nondestructive technique that allows obtaining a fully three-dimensional view of the bone structure. It is based on the same physical and mathematical principles as the medical CT scanner, but the big advantage of micro-CT lies in the use of a microfocus X-ray source so that much higher resolutions (up to 10 μm) can be achieved. Since individual trabeculae can be visualized at this resolution level, micro-CT has already been proven to be a valuable instrument in the characterization of trabecular bone structure.[5–9] Many authors reported very good correlations for different trabecular bone structural parameters between conventional histomorphometry and micro-CT[6,7,9]: for example, Müller et al[9] examined 63 human bone biopsies by means of histomorphometry and micro-CT (resolution, 14 μm) and calculated two-dimensional (2-D) and three-dimensional (3-D) parameters on the basis of the two methods. High correlations, (r values ranging from 0.84 to 0.93 for different parameters) were found. The mean percentage differences between histomorphometry and micro-CT ranged from 2.5 to 6.1%.

The use of micro-CT is limited to *in vitro* examinations: because of dosage considerations and because of size limitations of the object that can be scanned (field of view limitations), it will not be possible to achieve such high resolutions *in vivo*.[10] In this article, we will explore the possibilities of micro-CT in the *in vitro* characterization of bone structure around oral implants. First results will be presented for bone–implant specimens taken from animal experiments.

Furthermore, because micro-CT can present the full three-dimensional data of the trabecular bone structure around an oral implant in a digital format, these data can be used to create accurate finite-element (FE) models of the bone–implant system. Whereas in previous FE studies, trabecular bone was modeled as a continuum,[11] it will now be possible to model individual trabeculae and to study the stress transfer at the interface from the implant to individual trabeculae. The procedures to derive two-dimensional FE models based on micro-CT will be presented.

MATERIALS AND METHODS

The examined specimens were obtained from a previous animal experimental study conducted at the Dental School of the University of Nijmegen: grit-blasted and Ca-P magnetron sputter-coated titanium implants (length, 12 mm; diameter, 4 mm; Astratech implants, Sweden) were installed at the medial side of the left and right femoral condyle of adult sheep. After sacrifice (at 6 or 12 weeks), the femoral condyles with the implants were excised and sectioned into smaller blocks. The tissues were fixated with a 10% buffered formalin solution, dehydrated in an alcohol series, and embedded in methylmetacrylate. The specimens that we used for our study were all more or less wedge shaped, with one face of the wedge cutting through the implant in its longitudinal direction, so that each specimen contained only half an implant. We will further refer to this face as the reference plane.

Micro-CT imaging

The specimens were first scanned with the micro-CT system. For three specimens (specimen A, B, and C), CT images had already been taken, using a Skyscan 1072 microfocus CT scanner. Figure 1 gives a schematic overview of the micro-CT system: a polychromatic X-ray conical beam is produced by the microfocus X-ray tube, which has a focal spot of 10 μm (''small spot'') or 30 μm (''large spot''), depending on the CT scan settings. The object is mount-

TABLE 1

Spatial resolution levels for different specimen sizes for the Skyscan 1072 micro-CT system*

Object diameter (mm)	Skyscan 1072
2	8
4	16
8	31
10	39
20	78
40	156
60	234
100	—
130	—

*The resolution of 8 μm for a 2-mm-size object is theoretical because the resolution is limited by the focal spot size, which amounts to 10 μm (in case of ''small spot''). The resolution in one slice, in microns, is two times the pixel size.

TABLE 2

Different CT scanning settings applied to three specimens

Specimen	Voltage (kV)	Thickness of aluminum filters: Source and specimens (mm)	Thickness of aluminum filters: Specimen and camera (mm)
A	130	—	—
A	130	0.3	3
A	90	—	2
B	130	—	—
B	130	0.3	3
B	90	—	—
C	110	—	—

ed on a turntable that can be shifted in a vertical and horizontal direction, allowing an optimal positioning of the object with respect to the source. Transmitted X-rays are absorbed and transformed into light by a phosphorous screen. Light is then detected by a CCD camera, consisting of a 2-D 512×512 array of pixels. This digitized signal is further transferred to a Dual Pentium 200 MHz NT workstation, on which the projection data are recorded and reconstructed.

Unlike the medical CT scanner, the object is then rotated around a vertical axis (total rotational angle 180°) while the source and detector remain at a fixed position. The stepping rotational angle is typically 0.9°, resulting in 200 different projections. After the data acquisition, the image is reconstructed slice per slice (maximum 512 slices) according to a convolution back-projection algorithm. In the case of 200 projections, the acquisition time is typically 2 hours; the reconstruction time amounts to 1 hour.

The biggest advantage of micro-CT, compared with the medical CT scanner, is the much higher spatial resolution (defined as twice the pixel size), both in plane and out of plane; these are equal in case of micro-CT. The resolution is proportional to the geometric magnification, so that smaller objects, which can be positioned closer to the source, are imaged with a higher resolution than are larger objects. The values for spatial resolution, corresponding to different specimen sizes, are summarized in Table 1 in the case of the Skyscan 1072 micro-CT. The implant-bone specimens for this study were imaged with a resolution of 60 μm (pixel size and interslice distance 30 μm).

Special attention must be paid to the choice of the X-ray energy level. Because of the strong attenuating properties of titanium, the energy must be high enough in order to avoid complete attenuation of the X-ray beam by the implant. On the other hand, because of the much lower linear attenuation coefficient of bone tissue, the energy must not be too high so that X-rays will still be attenuated by the bone. An additional difficulty is caused by the presence of the embedding plastic: the energy level should be chosen so that an optimal contrast between bone and plastic is created. Because all these conditions are more or less conflicting, one must clearly make a compromise. In order to look for the optimal settings, the specimens were scanned at two different voltage levels (with a current of 300 μA). Furthermore, the influence of hardware filters was examined: aluminum filters were placed either between the X-ray source and the object in order to reduce beam-hardening artifacts or between the object and the detector, which leads to a reduction of scattering artifacts. The different combinations of applied voltage levels and filters are summarized in Table 2.

All specimens were positioned so that the reference plane of the specimen was perpendicular to the original CT planes of reconstruction. Because the sample holder of the micro-CT apparatus did not permit a highly accurate positioning of the specimen, small errors of a few degrees with respect to the exact perpendicular position were introduced. Only part (approximately 10 mm) of the total height of the specimens was scanned so that higher geometric magnifications could be obtained, resulting in an increase of the spatial resolution. For visualization of the CT images, two different software packages—Noesys Slicer T3D (Fortner Research) and Mimics (Materialise, N.V.)—were used: both are able to carry out 2-D reconstructions in an arbitrary plane and 3-D reconstructions, which are other advantages of the use of micro-CT.

Comparison with histological sections

In order to determine the accuracy of the micro-CT technique, histological sections were prepared for one of the three specimens, namely specimen A. Six sections were obtained parallel to the reference plane at 100, 500, 900, 1450, 1850, and 2500 μm of the reference plane. The last two sections do not contain implant material. A modified sawing-microtome technique[2,3] was used to prepare thin sections (10 μm), which were then stained with basic fuchsin and methylene blue. The sec-

tions were then digitized and compared with the corresponding micro-CT slice of specimen A, parallel to the reference plane. Because of the small angular error (with respect to the reference plane) in the positioning of the specimen during CT scanning and during sawing with the microtome, it was not easy to find exactly the same CT slice. However, the registration was accurate enough to perform a qualitative evaluation of the accuracy of micro-CT.

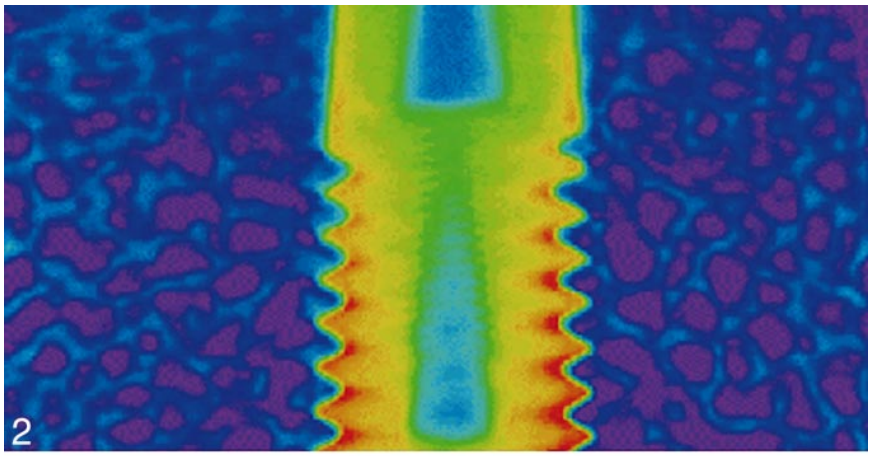
FE modeling

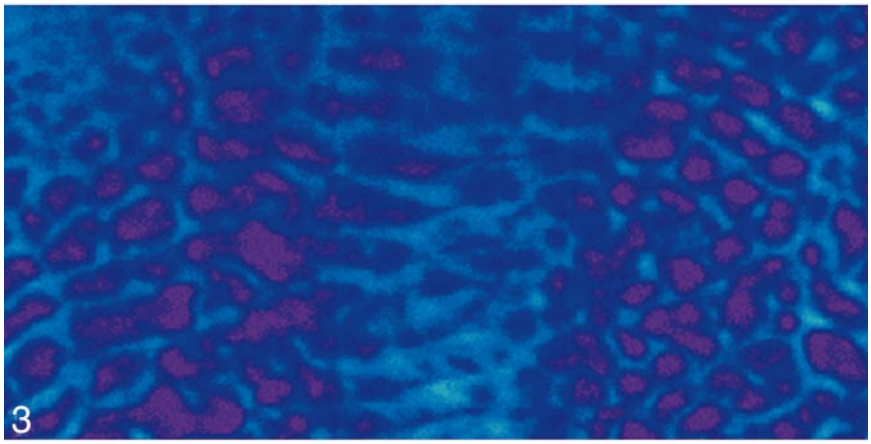
In order to construct individual FE meshes for the trabecular bone and for the implant, a segmentation must be performed to separate bone tissue from titanium and from the plastic. This is done by Mimics by specifying a minimum and a maximum threshold for the gray values, so that all pixels with a gray value between these thresholds will be assigned to bone. Once the segmented bone has been obtained, the program automatically calculates polylines that determine the perimeter of the trabeculae for each CT slice. The polylines in one slice are then used by the preprocessor of the FE program (MARC k7.2) to construct a two-dimensional FE model of the trabecular bone and the implant.

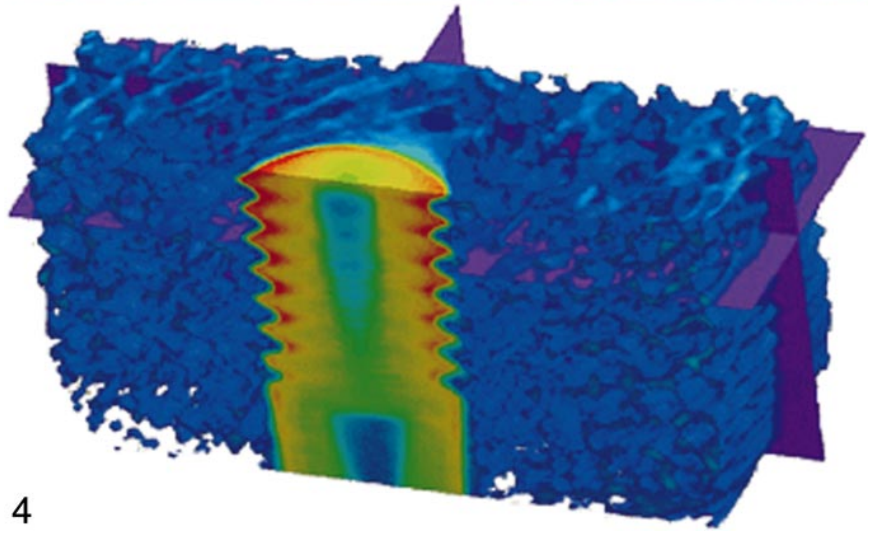
RESULTS

Micro-CT imaging

In Figs 2 and 3, two CT slices (for specimen A, scanned at 130 kV without filters) parallel to the reference plane are shown. The first slice (Fig 2) is taken through the implant; the second slice (Fig 3) is taken approximately 3 mm distal from the reference plane (*ie,* at approximately 1.5 mm distal from the implant). By means of the Noesys Slicer T3D software, the original 256 gray values were transformed in a color visualization (with 256 colors), each representing a different attenuation level: red corresponds to the highest attenuation, followed (in descending order of linear attenuation coefficient) by or-







FIGURES 2–4. Attenuation level is visualized by means of a color scale, with red being the highest attenuation and violet being the lowest attenuation. FIGURE 2. 2-D micro-CT reconstruction of specimen A in a plane through the implant, parallel to the reference plane. FIGURE 3. 2-D micro-CT reconstruction of specimen A in a plane parallel to the reference plane, at approximately 1.5 mm distal from the implant. FIGURE 4. 3-D micro-CT reconstruction of specimen A. The plastic is made invisible.

ange, yellow, light green, dark green, light blue, dark blue, and finally violet, which corresponds to the lowest attenuation. Individual trabeculae (shown in light or dark blue, depending on the bone tissue density) can be clearly distinguished from the plastic (in violet). From Fig 2, it can be seen that not all implant windings are filled with bone tissue. Because of beam-hardening effects, the titanium implant will not be reconstructed as one homogeneous material; instead, higher attenuation levels (red) are found at the implant perimeter, while lower levels (orange and yellow) are found at the inner part. Because we are clearly not interested in the visualization of the inner part of the implant, this does not present any substantial problems. Furthermore, it is interesting to notice the differences in trabecular bone porosity in Fig 3: in the middle of the image (*ie,* behind the implant), the apparent density looks much higher than at the left or right side of the image.

Another possibility of the visualization software is the creation of a three-dimensional reconstruction, as shown in Fig 4: here, the plastic was first segmented by means of thresholding and was made invisible. The three planes (in violet) define planes of two-dimensional reconstruction. In this case, they were chosen perpendicular to each other, but the images can be reconstructed in a plane with an arbitrary direction.

Different combinations of voltage and filters were evaluated in order to look for the optimal settings. For specimen A, the best contrast between bone and plastic was obtained with 90 kV in combination with the 2-mm Al filter between source and specimen. For specimen B, not much difference was noticed between 90 and 130 kV without filters, but the use of 130 kV in combination with filtering led to the worst image quality. It is, however, difficult to draw general conclusions from these preliminary results.

Comparison with histological sections

For specimen A, the six histological sections were compared with the corresponding CT slice. The CT data acquired with a voltage level of 90 kV (see Table 2) were used for this purpose because they had the best image quality. The results are presented for the histological section at 500 μm (see Fig 5). The corresponding CT slice is shown in Fig 6. Because the CT image only visualizes part of the total height of the specimen, the region of interest (ROI) must first be defined for the histological section. This can be easily done by using the implant windings as a reference. Because small errors in the exact positioning of the specimens could not be excluded during CT data acquisition and during section preparation, section and CT image will not exactly match. However, it is still possible to make a qualitative comparison of both techniques. From Figs 5 and 6, one can derive that the overall trabecular structure shows a good similarity: corresponding trabeculae can be easily identified in both figures. Even at the interface, there is a good qualitative resemblance in the number of implant windings that are filled with bone: from both figures, it is clear that at the left side of the implant, more windings are filled with bone tissue than at the right side. Furthermore, the titanium implant does not seem to produce any substantial artifacts: the only artifact due to titanium might be the occurrence of the small bright spots at the

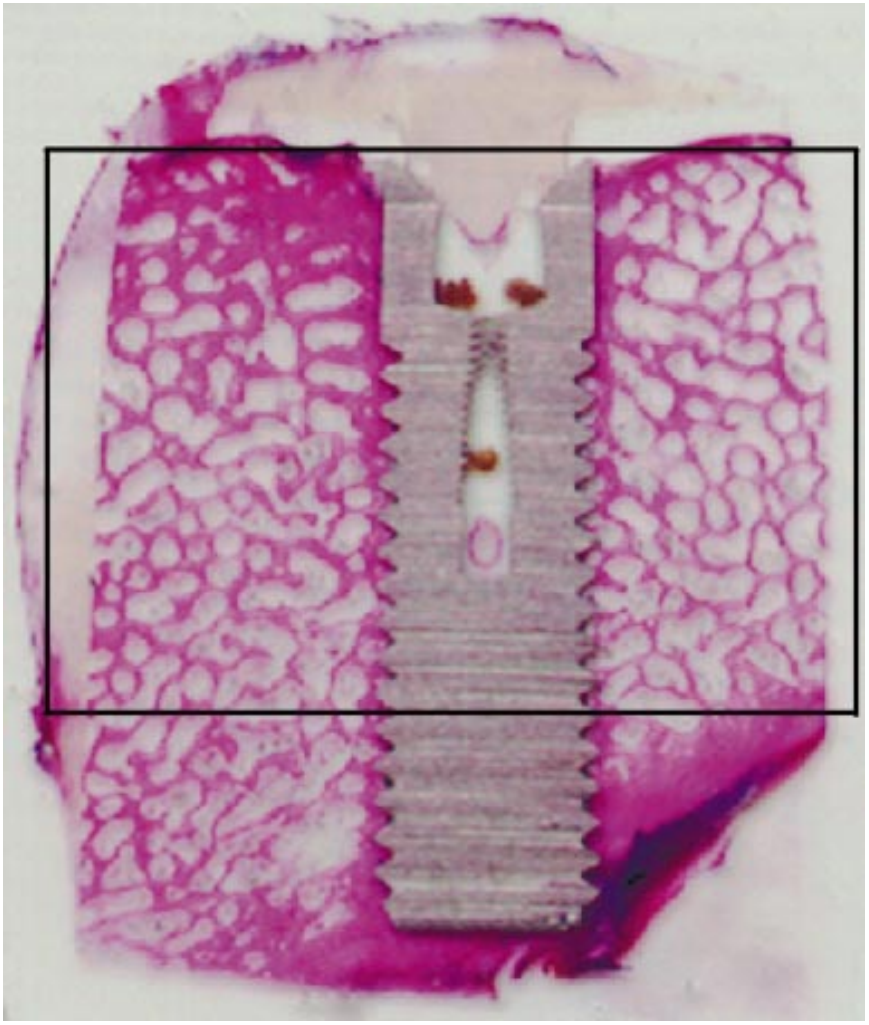


FIGURE 5. Histological section of specimen A taken at 500 μm from the reference plane. The rectangle represents the ROI, used for comparison with micro-CT.

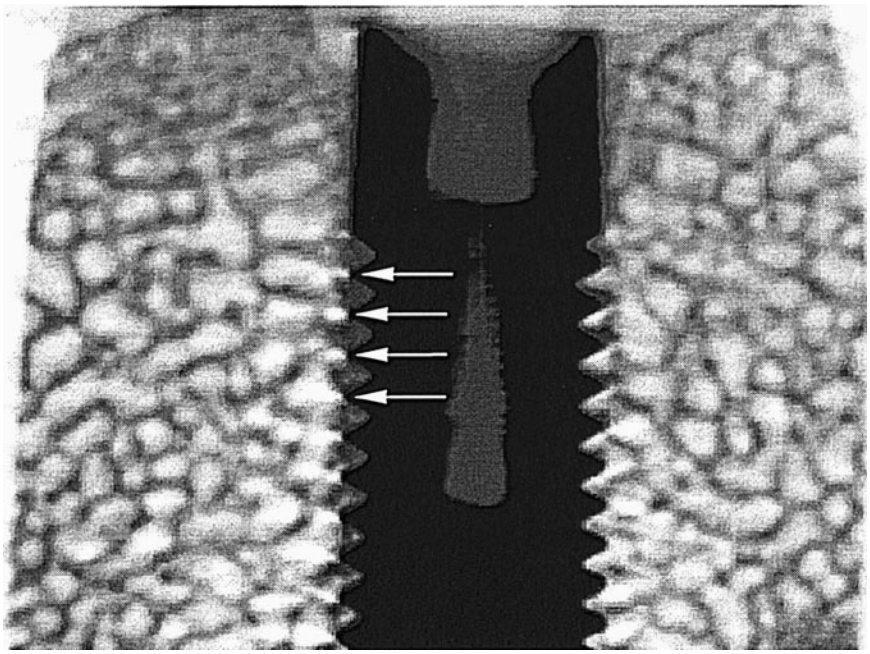


FIGURE 6. Micro-CT image of specimen A, corresponding to the ROI in Fig 6. The arrows indicate possible artifacts at the tips of the screw thread.

tips of the screw thread (indicated by the white arrows in Fig 6). At this moment, it is difficult to assess the micro-CT accuracy in a quantitative way because of the positioning errors. Further research is needed to answer this question.

The voltage level again plays an important role in the visualization of individual trabeculae. Comparing the data acquired with 90 and 130 kV, it was found that some small trabeculae that were clearly visible in the histological section and in the 90 kV image could not be detected when 130 kV was applied. On the basis of this result, it seems appropriate to scan the specimens with 90 kV instead of 130 kV.

FE modeling

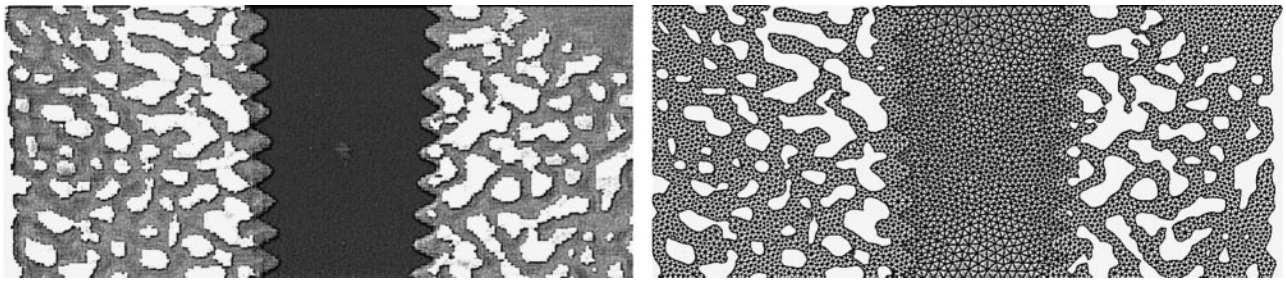
The procedure for deriving two-dimensional FE models from one CT slice is illustrated in Figs 7 and 8, respectively representing a CT slice through the implant and the corresponding FE model of the trabecular bone tissue and the implant. A Delaunay triangular mesher was used to construct a very fine mesh, consisting of 2639 elements for the implant and 7608 elements for the bone tissue. It is clear that to perform relevant FE analyses, it is necessary to scan the implant along the entire length. With this model, we only wanted to examine the feasibility of the procedure to create FE models from micro-CT.

DISCUSSION

The use of micro-CT for the evaluation of peri-implant tissues presents some attractive advantages in comparison to conventional histomorphometry: it is a fast, nondestructive technique that allows a fully three-dimensional characterization. In this study, we explored the possibilities of this new technique and examined its accuracy with respect to histological sections.

The presence of materials with such highly differing attenuation properties (titanium and bone) in one object causes substantial difficulties to obtain optimal image quality. Further research will be needed to optimize the acquisition settings (voltage, hardware filters, and so on) to minimize artifacts and to maximize the bone contrast.

Specimens used in this study were obtained from a previous animal experiment that was set up to investigate the influence of implant surface preparation on osseointegration. Therefore, specimen preparation that was carried out at the time of the animal experiment was not fully optimized for the micro-CT study. Specimens were already embedded in methylmetacrylate. Better contrast will certainly be obtained if specimens can be scanned that have not yet been embedded in plastic, because this significantly reduces the contrast and increases the possibility of accurately segmenting the bone tissue. The use of specimens containing only half of the implant was not dictated by the CT scan protocol but was another result of the previous specimen preparation. Although not



FIGURES 7, 8. FIGURE 7. Micro-CT slice parallel to the reference plane. A 2-D FE model was constructed for this slice. Contrast enhancement was achieved by means of windowing and edge enhancement (unsharp masking). FIGURE 8. Corresponding 2-D FE model of trabecular bone and implant for the slice in Fig 7.

performed as a function of this study, it resulted in the advantage that the mid-line section could be taken as the reference plane for comparing micro-CT with histological sections.

Although only a qualitative assessment of the micro-CT accuracy was performed, the first results look very promising. Good similarities were found between histological sections and corresponding CT slices with respect to trabecular bone structure and amount of bone at the implant interface. The influence of artifacts due to the presence of titanium seems limited. More research will, however, be required to evaluate the accuracy in a more quantitative way. We are now conducting a new animal experiment, for which the bone-implant specimens will be scanned before being embedded in plastic so that image contrast will be more optimal. These specimens will be suitable for a quantitative evaluation of the image accuracy. Results of this study can be expected in the near future. At this moment it is not clear whether micro-CT could discriminate between bone tissue and soft tissue at the interface in order to calculate the percentage bone contact. This depends not only on the spatial resolution (pixel size) but also on the resolution to detect small attenuation differences and on the effect of titanium on the CT reconstruction in adjacent regions. It is clear that the light microscope still has a much higher resolution, so quantitative measurements on histological sections will still have a higher accuracy.

The use of micro-CT in the characterization of bone structure around oral implants is also very interesting from a biomechanical viewpoint. With micro-CT–based models, individual trabeculae are modeled so that the load transfer from implant to bone can be more accurately calculated than with continuum-type models. Micro-CT can also be used for the validation of FE results. Animal experiments could be performed, where controlled loading conditions were applied to implants. Knowing the exact loading conditions, the corresponding mechanical stresses in the bone could be calculated by means of the FE method. After animal sacrifice, it would be highly interesting to evaluate the bone structure around the implant by means of micro-CT and to compare the observed bone structure with the calculated stresses (or other mechanical stimulus that leads to bone adaptation). In this way, micro-CT can be a tool for the validation of different bone adaptation theories.

Until now we have only created two-dimensional models of the trabecular structure around the implant. Because these models are based on only one CT slice, the exact connectivity and geometry of individual trabeculae can never be modeled in this way. This will strongly influence the calculated stress and strain distribution and will limit the validity of the results. In order to make full use of the advantage of a 3-D characterization of the bone structure—as is presented by micro-CT—one needs to construct 3-D FE models of the trabecular structure. Roughly, two different approaches to construct the 3-D FE mesh from micro-CT images can be distinguished, as described by Ulrich *et al*.[12] The first method, called the voxel-based method, converts a single voxel (or a group of voxels) into a hexahedral element. This method is very robust and is more easy to automate. However, it results in a steplike description of the bone surface and the implant–bone interface, which will bias the calculation of stresses and strains. A second method starts with the generation of a smooth, triangular bone (or implant) surface. In a second step, tetrahedrons are then constructed on the basis of these triangles to mesh the bone (or implant) volume. The advantage of the last method is that smooth bone and implant surfaces will be obtained. This makes this method more suitable for FE calculations of the load transfer from the implant to the bone. However, the algorithm to construct the tetrahedral mesh is much more complex than for the voxel-based method. The Division of Biomechanics at the KU Leuven is currently investigating different procedures to build such large-scale micro-CT–based FE models of implant-bone specimens.

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